14. (Twice Amended) An established cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, p-glycoprotein, alkaline phosphatase and γ-glutamyltransferase, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the cell obtained by treating brain capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

C5
CON1.

Amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (pages i - iv).

REMARKS

Claims 1-14 are currently pending in the application. Claims 1, 3, 5-7, 9-11 and 13-14 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Rejections Under 35 U.S.C. § 102(b)

Claims 1, 3, 6, 11 and 14 remain rejected under 35 U.S.C. § 102(b) in view of Greenwood et al. (*J. Neuroimmunol.* 71:51-63. 1996). The Office Action states that "the transport of substances such as drugs into the cells, inside-outside polarity of cells when cultured in vitro and the temperature sensitivity are all inherent properties of the cells disclosed by Greenwood et al.", and that "[t]he claiming of a new use, new function or unknown property which is inherently present in the prior art does not necessarily make the claim patentable."

The cells of Greenwood *et al.* and the cells of the present invention are different in that the Greenwood *et al.* cells are made by (1) isolating rat retinal cells, (2) transforming them using

a vector coding for a tsA58 T-antigen which is also associated with the G418 resistance gene as a selectable marker, (3) selection of transformed cells by growing in neomycin-containing media at 37°C, and (4) repeated subculture at 37°C to establish the neomycin-resistant cells that grow at 37°C (see, *e.g.*, page 53, right column, section 2.7).

The cells of the present invention, on the other hand, are made by (1) producing the vector pSVtsA58 ori(-)-2 by deleting the origin of replication (ori) from tsA58 T-antigen gene-integrated SV40, (2) purifying the tsA58 T-antigen gene by opening the vector pSVtsA58 ori(-)-2 with *Bam*HI, (3) introducing the tsA58 T-antigen gene into the nucleus of a fertilized ovum, (4) transplanting the fertilized ovum to a foster mother to obtain a transgenic rat, (5) extracting the retinal capillary endothelial cells from the rat, and (6) repeated subculture at 33°C to obtain established cells that grow at 33°-37°C (see, *e.g.*, page 13, line 20 to page 14, line 14), and stop growing at 39°C.

The cells of the present invention are therefore different from those of Greenwood *et al.* in that first, the Greenwood *et al.* cells grow only at 37°C, while the cells of the present invention grow at 33°C, and stop growing at 39°C (see, *e.g.*, page 15, lines 3-8, page 16, lines 11-13 and 22-25). Second, the Greenwood *et al.* cells contain a retroviral vector with a neomycin resistance gene under the control of a constitutive retroviral promoter. The cells of Greenwood *et al.* therefore express all of the gene products related to neomycin resistance during all stages of growth. The cells of the present invention do not contain such a gene, and so are more similar to naturally-occurring retinal cells than those taught by Greenwood *et al.* In addition, the expression of neomycin resistance in the Greenwood *et al.* prevents one from performing any subsequent transformation with the cells and using neomycin resistance as a selective marker. The cells of the present invention, on the other hand, can be used in such a way.

Applicants have amended the claims to recite that the cells' growth is inhibited at 39°C, and that the cells do not contain a heterologous antibiotic resistance gene. Applicants respectfully submit that the cells as claimed are sufficiently distinguished from those of Greenwood *et al.*, and respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 103

Claims 1-3, 6-7, 10-11 and 14 remain rejected under 35 U.S.C. § 103 in view of Rudland *et al.* (International Application WO 97/39117, 1997) and Greenwood *et al.* (U.S. Pat. No. 6,090,624, 2000), further in view of Roux *et al.* (*J. Cell. Physiol.* 159:101-113, 1994) and Villalobous *et al.* (*J. Pharmacol. Exp. Ther.* 282:1109-1116, 1997).

The previous Office Action stated that Rudland *et al.* does not teach "the immortalized choroid plexus cells with their transgenic rat", and Applicants noted in the Reply to that Office Action that one of the sections of this reference cited in that Office Action (page 32, lines 3-6) teaches cell lines derived from mammary glands, not brain, which were subsequently confirmed to stain for milk fat globule membrane (page 33, lines 9-14). Furthermore, the other section cited by that Office Action (page 39, lines 20-22) teaches primary cultures of brain cells, which were cultured in such a way as to cause cells other than neuronal cells to die out (page 39, last three lines), leaving cells which expressed neuronal markers. This section therefore teaches mammary cells and neuronal cells, not retinal capillary cells, choroid cells or brain capillary endothelial cells.

The present Office Action states that the rejection of record cannot be argued "by presenting a piecemeal analysis of the references and their alleged failure to teach the claimed invention." The Office Action presents several court cases to support the premise that one cannot show nonobviousness by attacking references individually where the objections are based on a combination of references. The Office Action then states that "the stated motivation for combining the teachings of the references is the teachings by Rudland et al. who teach that their invention is amenable to use in other tissues to generate conditionally immortal cell lines and the teachings of Greenwood et al. (1998) who teach the structural identity of these cell types and teach that they serve as models for studying the blood/central nervous system interfaces."

Applicants note that Rudland *et al.* teaches a rat cell line derived from a transgenic mammal, where the cell line comprises (1) a conditional oncogene or immortalizing gene, and (2) a cell type specific promoter (see, *e.g.*, Abstract; page 11, paragraph 1; claims, etc.). The

cells disclosed in Greenwood *et al.*'s U.S. Pat. No. 6,090,624, like Greenwood *et al.* 1998, above, contain an antibiotic resistance gene, unlike Applicants' cells.

Applicants maintain that the Office Action has failed to establish a *prima facie* case of obviousness under the requirements of 35 U.S.C. § 103(a), as set forth by the U.S. Court of Appeals for the Federal Circuit. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings (*In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)). Second, there must be a reasonable expectation of success. *Id.* The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on Applicants' disclosure. *Id.* Finally, the prior art reference (or references when combined) must teach or suggest *all the claim limitations*. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (C.C.P.A. 1974). Furthermore, the Court of Customs and Patent Appeals has held that "[r]eferences relied upon to support a rejection under 35 USC 103 must provide an enabling disclosure, i.e., they must place the claimed invention in the possession of the public." (*In re Payne, Durden, and Weiden*, 203 U.S.P.Q. 245, 255 (C.C.P.A. 1979)).

The Manual of Patent Examining Procedure (MPEP) reiterates this standard at § 2142:

> To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure.

(citing *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)). The MPEP also states that

> The initial burden is on the examiner to provide some suggestion of the desirability of doing what the inventor has done. "To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references."

(citing *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985)).

Applicants maintain that these requirements have not been met. The references cited in the Office Action, when combined, do not teach or suggest all of the claim limitations, or they require limitations that Applicants have found to be unnecessary. The cells of both Greenwood *et al.* references, for instance, contain an antibiotic resistance gene, and the cells of Rudland *et al.* require a cell type-specific promoter.

The present Office Action asserts that "[t]he expectation of success comes from Rudland et al. who teaches that their method is amenable to use in other tissues to generate conditionally immortal cells lines and Greenwood et al. who successfully teach the generation of conditionally immortalized cells ... and teach the structural similarity between their cell types and those used in the instant case." The Office Action goes on to state that "[i]t would be obvious for one of ordinary skill in the art to use the structurally similar cell types disclosed by Greenwood et al. and be motivated to generate conditionally immortalized cell lines using the methods of Rudland et al."

Applicants note that if one were to do as the Office Action suggests, one might produce cells with either a cell type-specific promoter (as taught by Rudland *et al.*), or an antibiotic resistance gene (as taught by the Greenwood *et al.* references), or both. Applicants note that one of ordinary skill in the art might produce such cells -- it is not at all clear that one there is a reasonable chance of successfully producing the cells of the present invention, especially given that they first require the production of a transgenic animal. Iin addition, Rudland *et al.*'s requirement of inclusion of a cell-type specific promoter adds a level of complexity (and unpredictability) not required for production of Applicants' cells.

Applicants therefore maintain that (1) combining the references to make the cells as presently claimed is not an obvious combination, but is merely a combination that is obvious to *try*, given hindsight knowledge of the present invention, and (2) combining the references in such a manner still does not produce the cells as presently claimed. Applicants therefore respectfully request that the rejection on this basis be reconsidered and withdrawn.

Applicants submit that in view of the foregoing remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over the claims of the present invention.

Respectfully submitted,

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MARKED-UP VERSION OF AMENDMENTS:

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Please amend claims 1, 3, 5-7, 9-11 and 13-14 as follows:

1. (Twice Amended) A conditionally immortalized cell established from a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene.

3. (Twice Amended) An established cell derived from retinal capillary endothelial cells, which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, and p-glyoprotein, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene.

5. (Twice Amended) A method of establishing a conditionally immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, and p-glycoprotein, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the method comprising treating retinal capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

6. (Twice Amended) An established cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, and p-glycoprotein, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the cell obtained by treating retinal capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

7. (Twice Amended) An established cell derived from choroid plexus epithelial cells, wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of Na^+-K^+ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of Na^+-K^+ ATPase in the apical side, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene.

9. (Twice Amended) A method of establishing a conditionally immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of Na^+-K^+ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of Na^+-K^+ ATPase in the apical side, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the method comprising treating choroidal epithelium tissues of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

10. (Twice Amended) An established cell which expresses a temperature sensitive SV40 large T-antigen gene, wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and shows localization of Na^+-K^+ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of Na^+-K^+ ATPase in the apical side, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, which is obtained by treating choroidal epithelium tissues of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

11. (Twice Amended) An established cell derived from brain capillary endothelial cells, wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, which expresses a temperature sensitive SV40 large T-antigen, GLUT-1 transporter, p-glycoprotein, alkaline photosphatase, and γ- glutamyltransferase, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene.

13. (Twice Amended) A method of establishing a conditionally immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, p-glycoprotein, alkaline phosphatase, and γ-glutamyltransferase, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the method comprising treating brain capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

14. (Twice Amended) An established cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, p-glycoprotein, alkaline phosphatase and γ-glutamyltransferase, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the cell obtained by treating brain capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.